REMARKS

Claims 1-32 are pending in the present application. Claims 10-31 have been withdrawn as being drawn to non-elected inventions. For the reasons stated in Applicants' previous response, the Examiner is requested to rejoin the withdrawn claims. Claims 1-9 are being examined. Claim 32 is new. Support for claim 32 is found on page 1, lines 24-25 of the present application. Claim 6 is amended to correct a spelling error. No new matter has been added by way of this amendment.

Rejections under 35 U.S.C. 102

Claims 1-3 are rejected under 35 U.S.C. 102(b) as being anticipated by Nakari-Setala *et al.*, (1997). Applicants respectfully traverse.

Nakari-Setala *et al.* (1997) disclose the isolation of the *hfb2* gene by heterologous hybridization and the isolation of HFBII, a hydrophobin protein, from *T. reesei* fungal spores by extraction with trifluoracetic acid/acetonitrile solution, and by bubbling from a lactose-based culture medium. Additionally, Nakari *et al.* (1997) teach that HFBII is found in submerged cultures when the fungus is grown, *inter alia*, on cellulose or lactose-containing media or other carbon sources, which usually promote extracellular hydrolase production. Moreover, Nakari-Setal *et al.* (1997) describe that *hfb2* expression is induced by N and C starvation, and by light; while a second hydrophobin, *hfbI,* is not expressed on media containing complex plant polysaccharides, including cellulose, xylan, cellobiose or lactose.

Conversely, independent claim 1 is drawn to a method for decreasing foam formation during cultivation of a microorganism by modifying the organism, such that at least one protein associated with foam formation is not produced in an essential amount. As is known in the art and described in the specification (see, e.g. page 1 at lines 11-31) foaming occurs when cultures

are shaken to aerate a culture, thereby improving production of a desired product. When little or essentially no foam is formed during the cultivation of a microorganism, foaming is not a problem. (See, page 8 at lines 5-7). Thus, the instant invention is drawn to reducing the problem foam that occurs during cultivation, by modifying a foam-associated protein to below an "essential amount." Nakari-Setala *et al.* (1997) fail to disclose this element.

The Examiner asserts that the Nakari-Setala *et al.* (1997) reference discloses that hydrophobins are associated with foam because hydrophobins are collected from foam formed in a cultivation medium upon bubbling. However, although Nakari-Setala *et al.* (1997) describe that the hydrophobin protein, HFBII, can be isolated from a lactose-based culture medium when air is bubbled into the medium with a Pasteur pipette, this finding does not disclose that HFBII is associated with the problem foaming of the instant invention. Moreover, foaming, as described in the specification, is associated with both aeration and agitation. "Bubbling the medium" is not associated with agitation.

Studies previous to that of Nakari-Setala *et al.* (1997), (as described in Nakari-Setala *et al.* ((1996), see IDS filed February 20, 2002)) to which Nakari-Setal *et al.* (1997) refer, reveal that in static cultures, the hydrophobin protein Sc3p aggregates in the cell walls of the aerial hyphae of *S. commune* as SDS-insoluble complexes. (See, Nakari-Setala *et al.* 1996, page 253, right column through to page 254, left column). In contrast, another hydrophobin, HFBI, is tightly bound to the cells walls of submerged mycelium as SDS-insoluble aggregates in vigorously shaken cultures. The HFBI is so tightly bound that a 60% ethanol solution can only partially liberate the HFBI protein from the cell walls. Although *S. commune* is not generally regarded as a production host and the static culture conditions used are not relevant to foaming, the *S. commune* studies, coupled with Nakari-Setala *et al.*'s (1996) observations of HFBI, caused the authors to conclude that "[d]uring cultivation in shaken flasks, the fungal mycelium is

vigorously aerated, which could lead to aggregation of [HFBII] in the cell wall in a similar way to what occurs on aerial hyphae." (See, Nakari-Setala *et al.* (1996), page 253, right column). Therefore, Nakari-Setala *et al.* (1996) disclose that hydrophobins such as HFBI, when cultured under conditions that are associated with foaming, *i.e.* agitation, are not generally secreted into the medium to result in foam formation, but are tightly bound to cell walls. Hence, the reference does not teach that sufficient amounts of HFBI are in the medium to allow a person of skill in the art to conclude that foam formation is associated with this protein.

In fact, Nakari-Setala *et al.* (1996) do not disclose any data at all regarding whether or not HFBI is present in an agitated and aerated culture medium. This reference only discloses that HFBI is present in a solution resulting from bubbling the medium, not the vigorous shaking typically associated with foam. Likewise, as stated above, Nakari-Setala *et al.* (1997) only disclose that HFBII is present in a solution resulting from bubbling the medium, and do not disclose the amount of HFBII in a vigorously aerated medium. Hence, there is no disclosure correlating HFBII and foam formation.

Moreover, in addition to failing to disclose the correlation between HFBII and foam formation, Nakari-Setala *et al.* (1997) do not disclose the element of reducing proteins to an essential amount. For example, the Nakari-Setala *et al.* (1997) reference does not assess how HFBII protein levels change in response to varying culture conditions. Their investigation is limited to the effect of culture conditions on mRNA levels. As is well known in the art, the correlation between mRNA and protein levels is often insufficient to predict protein expression levels from mRNA data. Therefore, Nakari-Setala *et al.* (1997) fail to disclose all of the elements of claim 1.

In order for a reference to anticipate a claim, each and every element of the claim must be disclosed either expressly or inherently in a single prior art reference. Because Nakari-Setala *et al.* (1997) do not disclose the element of reducing a foam-associated protein, Nakari-Setala *et al.*

(1997) do not anticipate claim 1. Because claims 2 and 3 depend on claim 1, these claims also are allowable at least by virtue of their dependency.

Rejections under 35 U.S.C. 103(a)

Claims 1-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nakari-Setala *et al.* (1997) in view of Wosten *et al.* and Spanu *et al.* Applicants respectfully traverse.

As described above, Nakari-Setala *et al.* (1997) do not disclose the element of claim 1 wherein the microorganism is modified in such a way that the microorganism does not produce an essential amount of at least one of the proteins, polypeptides or peptides associated with foam formation during cultivation.

Additionally, Wosten *et al.* do not disclose this element. Wosten *et al.* disclose that the Sc3p hydrophobin spontaneously assembles into an SDS-insoluble protein membrane on the surface of gas bubbles or when dried down on a hydrophilic surface.

The Examiner asserts, however, that because Sc3p assembles into an SDS-insoluble protein membrane on the surface of gas bubbles, Sc3p is a foaming agent under aerated conditions that are introduced by bubbling or shaken cultures. However, whether or not an agent is a foaming agent primarily depends on its ability to both lower water surface tension and to stabilize foam bubbles. Wosten *et al.*, do not disclose, teach or suggest that Sc3p is able to stabilize foam bubbles. In fact, they teach against this possibility. For example, Wosten *et al.* describe that a Sc3p-containing supernatant, under vigorous agitation in a blender, comes out of the liquid to form a solid precipitate that can be removed by centrifugation. (page 1568, right column, lines 5-8). Production conditions resulting in foam formation, such as those in bioreactor cultivations, are generally vigorously agitated as well. Hence, based on the Wosten *et al.* disclosure, if Sc3p is present in such a culture medium solid precipitates would form, and thus, Sc3p could not stabilize foam.

Moreover, the stability of Sc3p-coated bubbles in regard to chemical or physical treatments is not clearly described. For example, Wosten *et al.* do not disclose, teach or suggest the possible concentration or temperature dependency of Sc3p aggregation. Weston *et al.* do, however, briefly mention that Sc3p-coated gas bubbles collapse when, for example, vacuum is applied, (see, 1569, 3rd paragraph, lines 4-5), and disclose that Sc3p aggregation is prevented by the presence of lipid solvents or detergents (page 1571, right column, paragraph 3, lines 7-10). Thus, it is reasonable to conclude that in some cultivation conditions, even in shaken cultures, hydrophobins may not form aggregates. Hence, the Wosten *et al.* reference does not disclose, teach or suggest that Sc3p is a foaming agent.

Finally, Spanu *et al.* do not disclose, teach or suggest that hydrophobins are associated with foaming. Spanu *et al.* disclose the deletion of a hydrophobin gene in order to study which affect the deletion has on the pathogeniciity of a fungus to its host plant. Although the hydrophobin is disclosed as being secretable in a medium when grown in liquid culture, as described above, that finding is not sufficient to associate a hydrophobin with foaming. Additionally, Spanu *et al.* do not disclose teach or suggest whether or not the hydrophobin is associated with air bubbles.

In order to have a *prima facie* case of obviousness, the combined references must teach or suggest all of the elements of a claim. Because the combination of Nakari-Setala *et al.* (1997), Wosten *et al.* and Spanu *et al.* do not teach or suggest every element of claim 1, claim 1 is not obvious over these references. Because claims 2-9 depend on claim 1 and incorporate all of its elements, claims 2-9 are allowable at least by virtue of their dependency.

If the Examiner has any questions concerning this application, the Examiner is requested to contact Linda T. Parker, Ph.D., Reg. No. 46,046 at the telephone number of (703) 205-8000.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

In view of the above amendment, applicant believes the pending application is in condition for allowance.

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Respectfully submitted,

By _____
Gerald M. Murphy, Jr.
Registration No.: 28,977
BIRCH, STEWART, KOLASCH & BIRCH, LLP
8110 Gatehouse Road
Suite 100 East
P.O. Box 747
Falls Church, Virginia 22040-0747
(703) 205-8000
Attorney for Applicant